up to 6 Unipucks (96 samples). EMBL/ESRF pucks are also acceptable with a capacity of 30 samples. In addition, MXCube and ISPyB software platforms for data collection and sample tracking/experiment reporting are routinely used at the beamline, allowing automated centering and the possibility to download the results obtained with the EDNA automated data processing pipeline through a web browser (https://ispyb.cells.es/). Native data collection has been facilitated by a Helium chamber developed "in-house" providing initial promising results. The beamline allows "in-situ" diffraction and serial crystallography experiments have been carried out successfully. XALOC is continuously open to new proposals providing beamtime within a few weeks. Current possibilities and upgrades that will become available in the near future will be discussed.

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Single Molecule-Force Spectroscopy for Monitoring the Structural Changes of alpha-synuclein Induced by Ligands Binding

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The description and correct identification of the different conformations of heterogeneous proteins, such as intrinsically disordered proteins (IDPs), is a challenge in the structural biophysical, due to the impossibility of using the standard bulk techniques.
Despite this hindrance, some different approaches have been successfully employed in the characterisation of the heterogonous populations that coexist in solutions. Among these, a prominent role has been played by Native - Mass Spectrometry (N-MS) [1] and Single Molecule - Force Spectroscopy (SM-FS), by means of an Atomic Force Microscope [2,3].
In the present work, we combine a single molecule approach (SM-FS) to a very sensitive ensemble technique (N-MS) to probe conformational properties of alpha-synuclein (a-syn), an IDP whose misfolding and aggregation is the main cause of Parkinson Disease. Particularly, we investigate the structural changes driven by two different ligands binding the a -syn, Epigallocatechin-3-Gallate and Dopamine, both known to affect the fibrillation process in vitro.
As a result, both SM-FS and N-MS highlighted a conformational change of a-syn from an unstructured to a more compact (N-MS) or mechanically resistant (SM-FS) conformation induced by the ligands, anyway the two pictures are not completely superimposable. However, our comparative analysis indicates how the complementarity of the employed biophysical techniques can be exploited to characterize components of IDPs conformational ensembles.

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Structural analysis of the binding mode of choline-o-sulfatase provides essential clues on the substrate promiscuity of the phosphatase superfamily

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Choline-o-sulfatase (COSe, E.C. 3.1.6.6), is a member of the alkaline phosphatase superfamily hydrolyzing choline-o-sulfate into choline and sulfate [1]. This superfamily is widely studied due to its substrate promiscuity, since this feature is often correlated to conformational flexibility [2]. This is a highly desirable feature for Protein Engineering, and has prompted many structural studies on the molecular basis responsible for enzymatic promiscuity [3]. In this work, we report a deep structural and biophysical analysis on WT COSe and its C54S active-site mutant. We show for the first time the binding mode of this member of the alkaline phosphatase superfamily with i) the natural substrate choline-o-sulfate, ii) the natural products, sulfate and choline, and 3) an unexpected ligand (HEPES). Our results provide essential clues on the molecular determinants governing the activity of this enzyme, involving a different mechanism to that proposed recently [1].

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Insights into the mechanism of signalling in the Rcs system

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The Rcs system present in enterobacteria plays a crucial role in motility, capsule and biofilm formation upon sensing envelop integrity [1]. It is composed of an outer and an inner membrane proteins named RcsF and IgaA, respectively, involved in signal detection where IgaA is essential, and of the phosphorelay system RcsCDB that senses and transduces the signal to the cytoplasm [2]. The RcsCDB system comprises the hybrid histidine kinase RcsC, the phosphotransferase RcsD and the response regulator RcsB. While RcsC and RcsD are also bound in the inner membrane RcsB acts as a transcriptional factor to activate or inhibit expression of target genes. Upon stimuli detection by RcsF and IgaA, RcsCDB transduces the signal catalysing a phosphorelay cascade that starts with autophosphorylation of RcsC, followed by phosphotransfer to RcsD which in turn transfers the phosphoryl group to RcsB for its activation. RcsB is a master piece of the Rcs system controlling multiple genes either alone, inhibiting transcription of the *flhDC* flagellar master operon [3] or activating expression of the small regulatory RNA *rprA*, or interacting with auxiliary transcriptional factors such as RcsA to regulate the operon *wca* -also named *cps* involved in synthesis of colanic acid capsule [4] in a phosphorylation-dependant or independent manner [5]. Our goal is to decipher the molecular bases involved in the sensing and transducing mechanism of the Rcs system. For that purpose, we are producing all components of the Rcs system to conduct functional and structural studies. In that sense, we have recently solved several structures of RcsB from *S. typhimurium* that has allowed us to visualize the conformation competent for DNA binding and provide understanding on the phosphorylation mechanism [6]. With that information we have generated several mutant variants of RcsB that have been

characterized in vitro and in vivo to understand the activation mechanism. So far, our data proposes new regulatory pathways for this system.

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Biochemical and structural insights of membrane protein insertion and folding in bacteria: The holo-translocon

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In all organisms, many proteins must be transported across and into membranes before arriving at their functional destination. In bacteria, the SecYEG translocon is a protein-conducting membrane channel that can function in promoting the transport of secretory proteins or assisting the insertion of integral membrane proteins [1]. In this last case,SecYEG associates with YidC and SecDF-YajC to form a functional complex called the holo-translocon (HTL) able to insert and fold membrane proteins in a co-translational translocation mechanism universally conserved [2,3].
We have determined the unambiguous existence of the HTL as an intact supercomplex of SecYEG–SecDF–YajC–YidC in native bacterial inner membranes, by using SMALPs [4]. By in vitro cell-free translation/translocation analysis, we have monitored the membrane protein insertion and folding activity of the HTL and its individual components [4]. We have observed structurally by electron microscopy, SANS and molecular dynamics that the structure and function of the HTL highly depend on its lipid composition with functional implications in protein transport [5-8]. Finally, we have assembled and captured in vitro the ribosome in the act of co-translational membrane protein insertion through the holo-translocon, visualising this big and fundamental machinery by high-resolution cryo-electron microscopy.
The understanding of the membrane protein insertion machinery in bacteria will create the basis for the design of new antibiotics, creating new strategies for fighting against infections.

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Ornithine decarboxylase and its paralogues from Xenopus laevis: functional and degradative aspects

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Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the biosynthesis of polyamines, organic cations that play an important role in cell growth, proliferation and differentiation. In mammalian cells, ODC is a short-lived cytosolic protein that, in contrast to most proteins, is degraded by the proteasome without ubiquitination. In addition, ODC degradation is stimulated by binding to the protein antizyme (AZ1) and prevented by the presence of antizyme inhibitors (AZINs), protein homologues of ODC but devoid of enzymatic activity. On the contrary, the degradation of both AZIN1 and AZIN2 is decreased by binding to AZ1 and is ubiquitin-dependent. Most studies trying to understand the molecular basis of this discrepancy have been carried out using murine proteins. In the present work, we have analyzed some functional and degradative aspects of the *Xenopus laevis* orthologues *ODC1* and *ODC2*/AZIN2, by studying different properties of these proteins in a heterologous HEK293T expression system. Our results have shown that: 1) xlODC1 is a bona fide ornithine decarboxylase (Km^{Orn}= 0.023±0.006 mM); 2) xlODC2/xlAZIN2 is not an antizyme inhibitor but only a lysine decarboxylase (Km^{Lys}= 1.06±0.25 mM) responsible for the formation of the diamine cadaverine; 3) xlODC1 and xlODC2/xlAZIN2 are, as mouse ODC, short-lived proteins ($t_{1/2}$= 136 and 34 min, respectively); 4) AZ1 promotes the degradation of both proteins although their C-terminal sequences are different from that of mouse ODC; 5) whereas the truncation of the 21 amino acid residues of the C-terminal region of xlODC2/xlAZIN2 prevented its AZ1-dependent degradation, the substitution of this region for the corresponding C-terminal region of mouse AZIN2 did not, suggesting that the influence of this region in the degradation of ODC paralogues is dependent on the rest of the protein. According to all these results, in *Xenopus laevis*, it does not appear to exist an orthologue of the mammalian AZIN2 and, consequently, the term xlODC2 should be exclusively used.

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Archaeal Like-Sm proteins: Computational analysis and functional modelling

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Like-Sm (LSm) proteins are key components in central RNA metabolism, representing a conserved family from an evolutionary point of view. Although a long list of bacterial and human studies have been performed, there is little information about archaeal molecular phylogeny. Current study attempts to figure out these relationships by applying biocomputational techniques to identify physicochemical properties and highly-conserved regions. At least one species from each genus of the *Archaea* domain was selected, analysing more than 150 LSm proteins of 109 different archaeal species. Multiple physicochemical properties such as molecular weight, isoelectric point, extinction coefficient, instability index, aliphatic index,